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First evidence of resistance to macrocyclic lactones, in *Psoroptes ovis* sheep scab mites in the UK

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Abstract

Ovine psoroptic mange (sheep scab) is an infection of substantial economic and animal welfare concern in the UK. Its prevalence has increased rapidly over the last 20 years and management is dependent on a small number of acaricidal compounds, many of which are also used to control a range of other endo- and ectoparasites. Here, the effects of the macrocyclic lactone (ML) moxidectin was considered using *in vitro* assays against mites from four farm populations where persistent treatment failure had been reported: two in west Wales, one from the England/Wales border and one in Herefordshire. The data demonstrate resistance in mites from all four farms. This is the first quantitative evidence of ML resistance in *Psoroptes* mites in the UK. Given the similarities in their mode of action it is highly likely that cross-resistance across the range of this class of compound will be found. The development of resistance to moxidectin is of considerable concern given the already high prevalence of scab infection in some regions; major difficulties in scab management should be anticipated if ML resistance becomes widely established in the UK.

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Introduction

Ovine psoroptic mange (sheep scab) is caused by an acute hypersensitivity reaction to the parasitic mite, *Psoroptes ovis*, leading to dermatitis, intense pruritus, self-trauma, weight loss and, in some cases, mortality (Roberts and others 1971). Immediately prior to its deregulation in the UK it was compulsory to treat all sheep prophylactically and, by 1988 when twice yearly immersion dipping was enforced, there were fewer than 40 reported outbreaks per year (French and others 1999). Following deregulation in 1992, many farmers abandoned prophylactic treatment, particularly with organophosphate insecticides (French and others 1994; Bisdorff and others 2006). Over the subsequent 20 years, the prevalence of scab increased substantially, while showing strong regional variation, with an average of 13.9% of flocks reporting at least one outbreak per year in Scotland, Northern England and Wales (range 7.1-20.5%) compared to 5.2% of flocks reporting at least one outbreak per year in Central, East and South West England (range 3.3-6.4%) (Bisdorff and others 2006; Rose and Wall 2012). The Sheep Scab (Scotland) Order 2010 once again made the disease notifiable in Scotland.

Regional differences within the UK have been attributed to the greater use of common grazing in upland areas since unrestricted mixing of animals facilitates transmission from infected to uninfected animals and makes prompt disease management more difficult (Rose and Wall 2012). Modelling analyses have demonstrated that in the prevailing economic conditions the use of prophylaxis is not a cost-effective scab management strategy for farmers, particularly for lowland sheep in areas where infection risk is relatively low (Nixon and others 2017). However, for farmers in upland areas, where infection risk is high, routine prophylaxis is cost-effective and an important welfare consideration.

There are two primary prophylactic treatments for scab prevention currently licensed in the UK: a long-acting injectable formulation of the macrocyclic lactone (ML) moxidectin and the organophosphate (OP), Diazinon, which must be used as a total-immersion plunge dip (Sargison and others 2007). When used as a preventative measure, a single injection of long acting 2% moxidectin can provide protection for up to 60 days (NOAH 2014). Diazinon plunge dip, confers protection for up to 63 days (Kirkwood and Quick, 1981). A range of other macrocyclic lactone products, such as doramectin or ivermectin, with relatively shorter residual activity, or Diazinon organophosphate dip can also be used reactively to treat scab. However, recent anecdotal reports of persistent treatment failure after the use of macrocyclic lactones have increased, particularly from Wales and the Welsh borders. The aim of this study, therefore, was to investigate whether such reports might indicate the initial stages of the development of resistance to macrocyclic lactones in the UK.

Methods and Materials

A laboratory bioassay was developed based on that described by Brimer and others (1995). Test plates were produced by filling sterile Petri-dishes (internal diameter 85 mm) with approximately 20 ml of sterile agar solution (agar base 40 g/l and 5% v/v of normal sheep serum) containing 1 ml of the macrocyclic lactone moxidectin (Cydectin 2% LA (Long Acting) Injection for Sheep, Zoetis Animal Health) diluted to varying concentrations in 100% ethanol, which were added to the agar solution just prior to the casting of the dishes to create concentrations of 500, 1000 or 2000 ng/ml. Negative control plates were made up by adding 1 ml of 100 % ethanol only to the agar solution in place of the moxidectin.

Psoroptes mites were derived from two sources. First, mites were collected from infested sheep maintained at the Moredun Research Institute (MRI). These sheep had never been treated for scab and the mite population had been in continuous passage, without exposure to acaricide, for at least 10 years. Second, *P. ovis* were collected from scab outbreaks; two in west Wales (Ceredigion and Camarthenshire), one from the Wales/England border (Hay-on-Wye) and one from England (Leominster, Herefordshire). Sheep on these farms had all been treated previously with various injectable acaricides but had failed to respond to treatment showing persistent signs of *P. ovis* infestation. Mites were removed from samples of wool and skin scrapings collected by the flock owner or veterinary surgeon.

Following collection and prior to experimental use, mites were held at 6 °C and were used within 3 days of collection. A maximum of 10 mites were added per Petri-dish at the centre of the agar; adults, tritonymphs or protonymphs were not distinguished; larvae were not used. Petroleum jelly was applied to the rim of the Petri-dish and Parafilm was used to seal it to prevent the mites from escaping. After sealing, the Petri-dishes were placed into an incubator at 80 % relative humidity and a temperature of 20 °C. Mites were inspected under a dissecting microscope every 24 hours and were considered dead if they demonstrated no movement after mechanical stimulation for 15 seconds with a fine paintbrush.

Large numbers of mites could be collected from the MRI infested animals, so the full range of moxidectin concentrations could be tested. MRI mites exposed to agar containing ethanol only are described as negative controls (MRI-) and MRI mites exposed to agar containing moxidectin are described as positive controls (MRI+). For all tests with MRI mites, there were three replicates for each moxidectin concentration used and the entire test was repeated twice at a four month interval,

with two independent collections from infested MRI sheep; data were then pooled for analysis. The outbreaks from west Wales and Leominster yielded relatively small numbers of mites and so these were exposed to only a single concentration of moxidectin of 500 ng/ml. The scab outbreak from Hay-on-Wye yielded larger numbers of mites and these were exposed to moxidectin at 1000 ng/ml and 2000 ng/ml.

The percentage mortality in each Petri-dish was ARCSIN transformed to normalise the variance before means were compared using one-way ANOVA with Tukey post-hoc tests.

Results

The mites collected from MRI and exposed to ethanol/agar only (MRI-), showed a gradual increase in mortality over 120 hours, reaching approximately 60% mortality by 120 hours after exposure (Fig. 1). When mites from MRI were exposed to agar containing 500ng/ml of moxidectin (MRI+), they showed a significantly higher rate mortality than the negative controls (MRI-), reaching approximately 90% mortality after 120 hours ($df=2$; $F=4.9$; $P<0.05$, Fig 1), showing that MRI mites were highly susceptible to moxidectin. In contrast, when exposed to 500 ng/ml of moxidectin the mites from the three field outbreaks (west Wales and Leominster) showed low levels of mortality, similar to that seen in the MRI mites exposed to ethanol only (MRI-) ($df=2$; $F=6.8$; $P>0.05$, Fig 1).

When exposed to moxidectin at 2000 ng/ml and 1000 ng/ml, positive control (MRI+) showed very high rates of mortality, approaching 100% after 72 h (Fig. 2). In contrast mites from the field outbreak from Hay-on-Wye showed low levels of mortality, with only 20-30% mortality after 120 h. This was significantly lower than that observed with the MRI mites (MRI+) exposed to similar concentrations of moxidectin ($df=2$; $F=32.7$; $P < 0.05$, Fig 2).

Discussion

When large numbers of sheep are treated with a small number of acaricides or insecticides the development of resistance is almost inevitable (Bates 1998). Resistance in *P. ovis* to synthetic pyrethroids was first confirmed in the UK in 1995 (Synge and others 1995) and in 1996–1997 there were at least 20 cases of suspected pyrethroid resistance detected (Coles 1998). The first case of resistance to the organophosphate propetamphos was described in 1996 (Clark and others 1996). Of particular concern in the UK with regard to resistance development is the fact that the same class of ML compounds is heavily relied upon for the control/treatment of a wide range of ecto- and

endoparasites, thus increasing the rate of application and exposure and inadvertently accelerating the rate of selection for resistance across a range of parasite taxa (Sargison and others 2007).

Here, four field populations of mites were collected from flocks which had shown persistent treatment failure to ML treatments. Analysis of sheep plasma 0.5 days after treatment with subcutaneous injection of moxidectin suggests that a plasma concentration of approximately 10-20 ng/ml might be expected (Lloberas and others 2013). While this was used as a guide to the dose range used here, the concentrations of moxidectin required to achieve mortality rates that were significantly different to that of the MRI-derived negative controls, were considerably higher (> 500 ng/ml). The manner in which the bioassay used affects the mites is therefore unclear; the mites may have ingested the top layer of agar or they may have been absorbing the active ingredient through the cuticle, but given the relatively high concentration required to achieve mortality, the latter is more likely.

Regardless of its precise mode of action, the data presented strongly suggest that treatment failure is likely to have been due to acquired resistance; the assay demonstrated good levels of mortality at concentrations of 500 ng/ml and above in MRI mites that had not previously been exposed to acaricides, but exposure to this concentration failed to give equivalent levels of mortality in all four field populations. This is the first demonstration of moxidectin resistance in *Psoroptes* mites in the UK. Given the similarities in their mode of action as GABA agonists, stimulating the binding of neurotransmitter and inducing paralysis in arthropods and nematodes (Nakao and Banba 2015), it is highly likely that cross-resistance across the range of this class of compound will be detected. Current work is underway to test this conclusion and more precisely identify a discriminating dose.

The development of resistance to MLs is of considerable concern given the already high prevalence of scab infection in some regions. Psoroptic mange is widespread in cattle in Belgium, affecting the Belgian Blue breed in particular. Treatment failures following injectable ivermectin suggest that ML resistance is widespread in the Belgian population of mites, resulting in great difficulty in its effective management (Lekimme and others 2010); a difficulty also encountered when cattle mange was introduced into the UK (Mitchell and others, 2012). This highlights the potential problems that are likely to be encountered should ML resistance become more widely established in sheep scab mites in the UK, leaving very few options for its effective management.

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Figure Legends

Fig 1. The percentage mortality of *Psoroptes ovis* mites over time (hours) exposed in an *in vitro* bioassay to moxidectin in agar at 500 ng/ml (circles) or ethanol in agar only (squares). Mites were derived either from three farm outbreaks (Red lines: Leominster, Ceredigion or Carmarthenshire) with known histories of treatment failure or from a culture at the Moredun Research Institute (MRI) with no history of acaricide treatment (black lines). The MRI mites were exposed to agar/moxidectin to provide positive controls (MRI+) or ethanol/agar only to provide negative controls (MRI-).

Fig 2. The percentage mortality of *Psoroptes ovis* mites over time (hours) exposed in an *in vitro* bioassay to moxidectin in agar at 1000ng/ml (circles) or 2000ng/ml (diamonds) or ethanol only controls (squares). Mites were derived either from one farm outbreak (Hay-on-Wye) with a known history of treatment failure (red lines) or from a culture at the Moredun Research Institute (MRI) with no history of acaricide treatment (black lines). The MRI mites were exposed to agar/moxidectin to provide positive controls (MRI+) or ethanol/agar only to provide negative controls (MRI-).

Figure 1

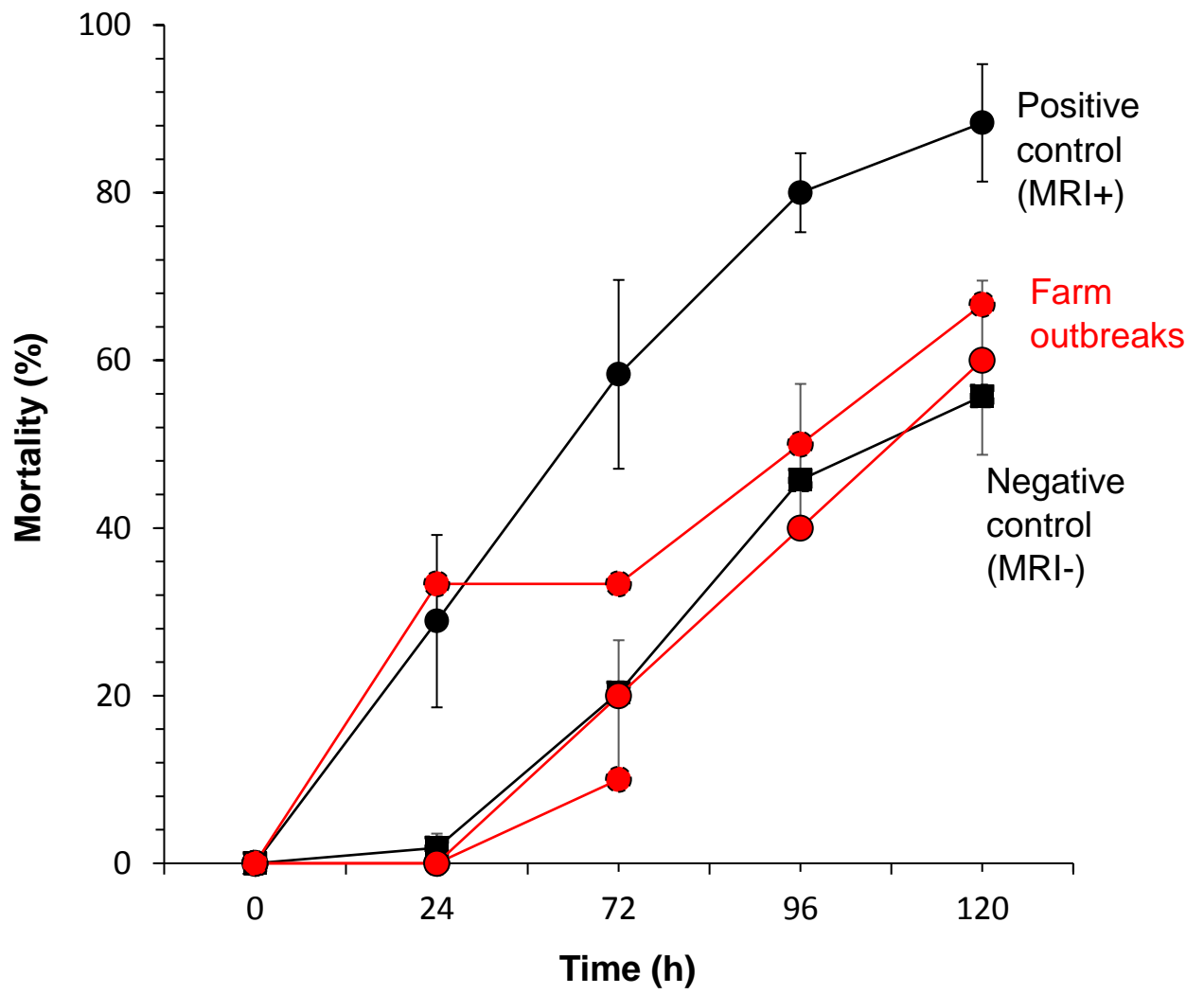


Figure 2

